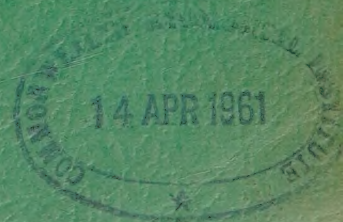

THE PHILIPPINE AGRICULTURIST

JOURNAL OF THE COLLEGE OF AGRICULTURE
AND CENTRAL EXPERIMENT STATION
University of the Philippines

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THE INFLUENCE OF COLD STORAGE CONDITIONS ON THE VIABILITY OF RICE AND CORN SEEDS ¹

R. C. DUMLAO and M. B. PARKER

WITH THREE TEXT FIGURES

The longevity of rice and corn seeds can be markedly prolonged by storing them at a mean temperature of 59°F (15°C) and a mean relative humidity of 59.5 per cent, which are lower than ordinary room conditions by 20.0°F and 21.1 per cent, respectively. Dormancy period of rice seems to be extended. Under ordinary storage conditions, varieties of the same crop have different rates of total loss of germination and deterioration of viability is very rapid after the critical period.

The maintenance of the viability of rice and corn seeds in storage is of practical and economic importance to farmers, seedsmen, and plant breeders because planting materials can be had over a considerable number of planting seasons without repeated maintenance plantings, the usual way of getting such valuable stocks. Without adequate storage facilities, seeds deteriorate rapidly and are of no value for commercial planting. Under ordinary conditions, farmers can keep the viability of their seeds to a maximum of only one year.

Keeping seeds in sealed containers with calcium oxide or powdered layer of charcoal may prolong their longevity under room temperature (San Pedro, 1936; Barton, 1935, 1939, 1941, and 1953; Rodrigo, 1952). However, sealing would be limited to a few stocks of seeds. Besides, this method would be impractical because exact amounts for one planting or one purpose have to be sealed to avoid withdrawing samples from another container and thus prevent the entrance of water vapor into the dry sealed seeds.

In general, longevity is markedly extended by low temperature and low humidity (Beattie and Boswell, 1939; Boswell *et al.*, 1940; Akamine, 1943; Toole, 1953).

Barton and Garman (1946) proved that sealed tomato seeds in cold storage could maintain a high viability after 13 years. Slatter (1950) said that the ideal temperature for storing dry seeds in air-

¹ Central Experiment Station Contribution No. 2057.

tight containers is between 23° and 40°F, or -5° and 5°C. In Sweden, Weibull (1952) found that vegetable and flower seeds kept in a commercial cold-storage plant operating at -20°C with a relative humidity of 85 to 95 per cent gave a germination as high as or equal to their original germination after four years. However, Sengbusch (1955) noted variable longevity of some kinds of seeds. Hemp and rye seeds stored at 20°C lost their viability after three years but when stored at -20°C, they retained their high germinative power; sugar beet maintained a high viability at both temperatures (-20° and 20°C). Foster (1946)² concluded from his compilation of literature on storage of vegetable seeds that beet, eggplant, and tomato seeds are generally more resistant to deterioration than sweet corn and onion seeds under unfavorable conditions. No doubt, different crops differ in their physiological state, inherited qualities and chemical composition, hence, their differing longevity period under the same conditions. It is apparent that they also differ in their life span and response to the same storage conditions.

At present, many improved and purified rice and valuable stocks of corn are included in the breeder stock of the Rice and Corn Improvement Program. These seeds are now kept in the cold storage room in the Division of Plant Breeding to prolong their viability.

It is therefore important to determine the length of time during which rice and corn seeds could retain high viability when stored at the cold storage wherein temperature and humidity are controlled.

MATERIALS AND METHODS

The experiment was started in January, 1957, when a cold storage room operating with a mean temperature of 59°F and a mean relative humidity of 59.5 per cent was installed at the Division of Plant Breeding. The materials, the initial moisture contents of which were reduced to 11.5-13.2 per cent were:

(1) Three Seed Board rice varieties harvested at the same season:

- a. Azucena, an upland variety
- b. Seraup kechil 36 Strain 482, a seasonal lowland variety
- c. Peta, a non-seasonal, lowland variety

² William Foster, "Maintenance of Viability of Vegetable Crop Seeds in Storage," 1946. Unpublished.

(2) Corn:

- a. Philippine Hybrid No. 1, a yellow flint corn hybrid
- b. Hawaii Yellow Sweet, a variety of sweet corn
- c. Laguna Popcorn, a variety of popcorn

To have a valid comparison, two groups of experiments with four replications each were conducted. The first group consisted of seeds kept at ordinary room storage conditions where the monthly mean range of temperature and of relative humidity were 77.0 to 85.1°F and 70.3 to 85.5 per cent, respectively. The other group was kept in the cold storage room operating at a monthly mean temperature and relative humidity range of 47.9 to 74.6°F and 47.0 to 66.9 per cent, respectively (fig. 1). Each group consisted of a half

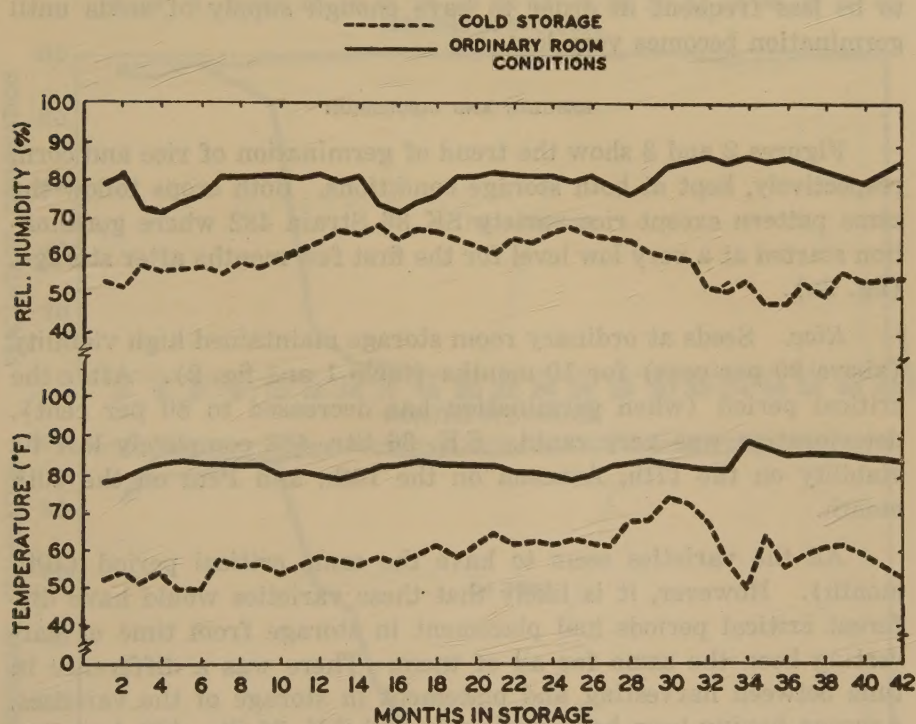


Fig. 1.—Mean monthly temperature and relative humidity at the storage places.

cavan of each variety. A germination test for corn was conducted one month after the start of storage and for rice, two months after the start of storage. One hundred seed samples from each repli-

cate were used in testing for percentage viability at bimonthly intervals. However, germination tests of those kept under ordinary room conditions were more frequent: when viability dropped by 10 and 20 per cent, monthly and biweekly determinations, respectively, were conducted thereafter.

Seeds were germinated in petri dishes with moist tissue paper and germination counts were taken a week after. The mean germination percentage of each determination was computed.

Except sweet corn which got lost, germination tests of the varieties kept in cold storage will be continued at five to six months intervals after the last determination reported here, to determine how long longevity is preserved. The interval of germination is planned to be less frequent in order to have enough supply of seeds until germination becomes very low.

RESULTS AND DISCUSSION

Figures 2 and 3 show the trend of germination of rice and corn, respectively, kept at both storage conditions. Both crops follow the same pattern except rice variety SK 36 Strain 482 where germination started at a very low level for the first few months after storage. (fig. 2a).

Rice. Seeds at ordinary room storage maintained high viability (above 90 per cent) for 10 months (table 1 and fig. 2). After the critical period (when germination has decreased to 80 per cent), deterioration was very rapid. S.K. 36 Str. 482 completely lost its viability on the 17th, Azucena on the 16th, and Peta on the 20th month.

All the varieties seem to have the same critical period (10th month). However, it is likely that these varieties would have different critical periods had placement in storage from time of harvesting been the same for all of them. There was a difference in time between harvesting and placement in storage of the varieties, Azucena having been harvested early and S.K. 36 Str. 482, late; but all three were placed in storage at the same time

The very low germination of S.K. 36 Str. 482 during the first few months of storage could be attributed to its dormancy. Peta and Azucena were no longer dormant at the first viability test. The dormancy period of S.K. 36 Str. 482 was extended to six months

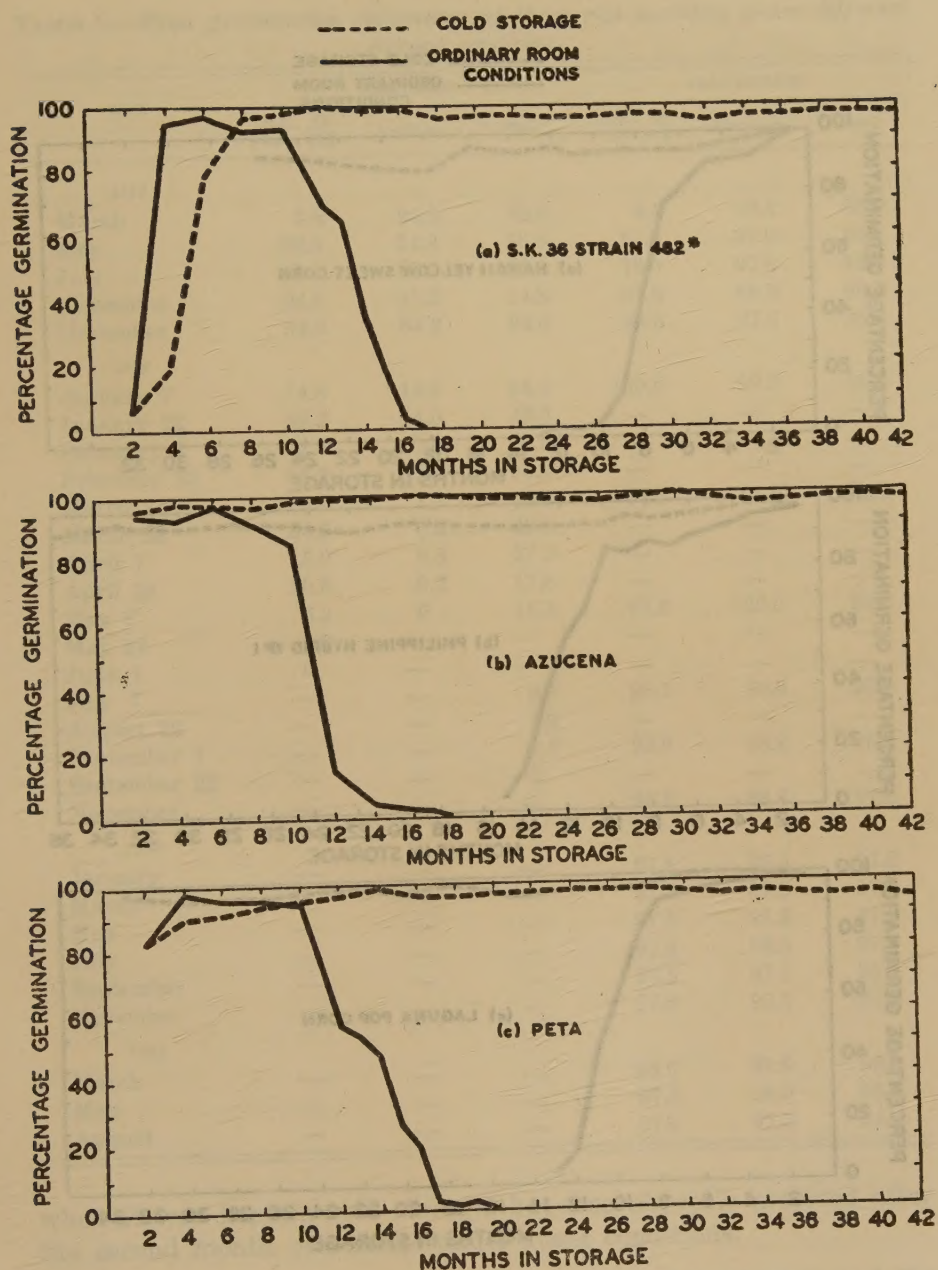


Fig. 2.—Viability of three rice varieties stored in cold storage and under ordinary room conditions. (The varieties were not placed on storage simultaneously after harvest).*

* Still dormant a few months after storage.

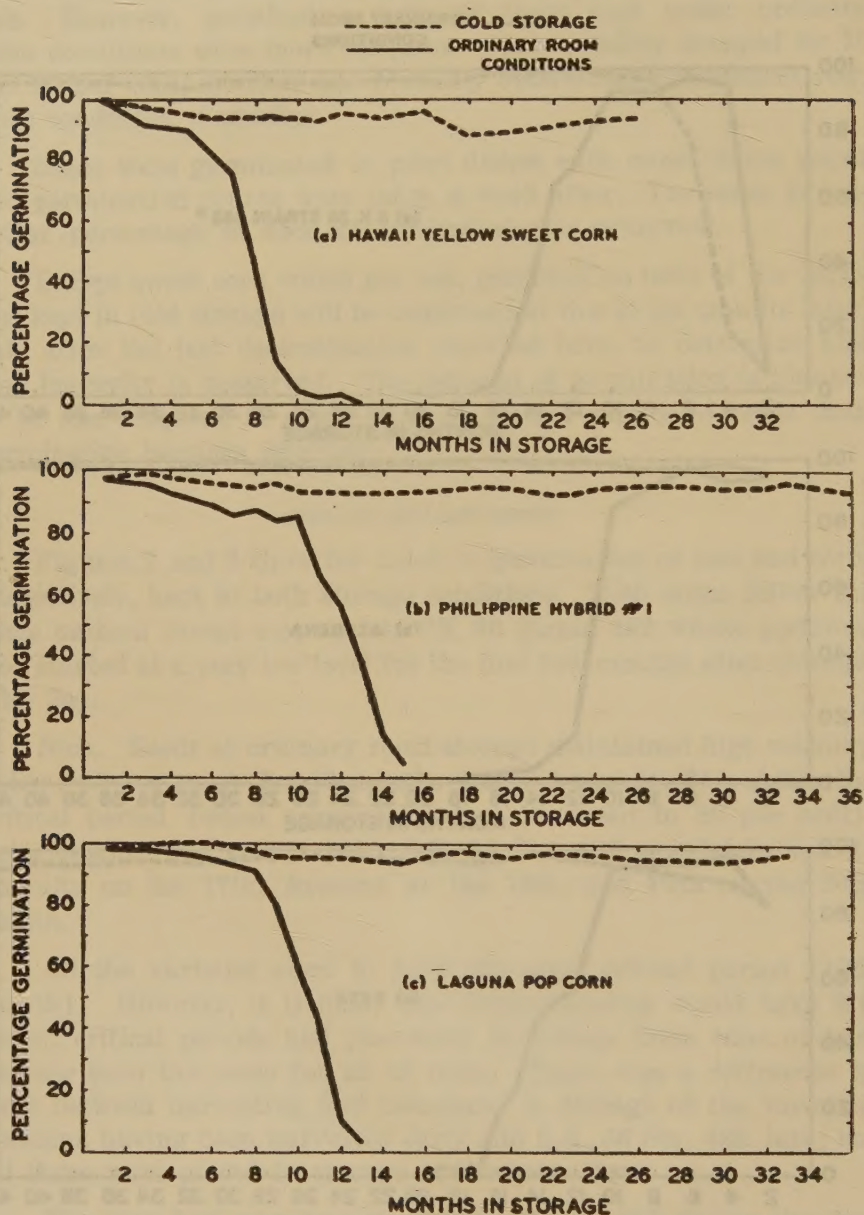


Fig. 3.—Viability of seeds of three types of corn stored in cold storage and under ordinary room conditions. (The corn types were not placed in storage at the same month).

TABLE 1.—Mean germination percentage of three rice varieties under different storage conditions

DATE	SEED ROOM			COLD STORAGE		
	S.K. 36 Str. 482	Azucena	Peta	S.K. 36 Str. 482	Azucena	Peta
<i>1957</i>						
March	3.2	95.8	82.5	3.5	96.2	82.0
May	96.5	94.8	98.5	21.0	97.0	90.8
July	99.2	98.0	95.2	78.0	97.8	92.5
September	94.2	91.2	94.8	97.0	96.5	93.8
November	94.0	84.8	94.0	98.5	97.5	95.0
<i>1958</i>						
January 7	74.8	16.5	64.2	100.0	99.2	96.8
January 22	69.2	14.0	49.0	—	—	—
February 7	72.5	7.0	50.8	—	—	—
February 22	56.2	1.0	53.2	—	—	—
March 7	56.2	1.2	50.3	98.8	98.0	98.2
March 22	54.5	0.8	49.5	—	—	—
April 7	15.0	0.8	37.5	—	—	—
April 22	20.8	0.2	17.8	—	—	—
May 7	6.2	0	16.5	99.8	100.0	95.5
May 22	0.2	—	—	—	—	—
June 7	0	—	—	—	—	—
July 7	—	—	0.8	96.7	99.0	95.5
August 22	—	—	1.2	—	—	—
September 7	—	—	0.5	98.0	98.8	96.8
September 22	—	—	0	—	—	—
November	—	—	—	98.2	98.5	97.0
<i>1959</i>						
January	—	—	—	97.5	97.5	97.2
March	—	—	—	97.2	97.2	97.2
May	—	—	—	97.8	97.5	97.2
July	—	—	—	97.8	98.0	97.0
September	—	—	—	96.5	97.5	95.5
November	—	—	—	97.0	95.5	96.0
<i>1960</i>						
March	—	—	—	98.0	97.0	95.2
May	—	—	—	97.5	98.0	95.0
August	—	—	—	97.5	97.5	94.2

when it was kept in cold storage, but was abruptly arrested after the second month of storage under room conditions.

Table 1 also shows the mean germination percentage of the seeds kept in cold storage. Seeds remained highly viable at almost 100 per cent during the last viability test (42nd month).

Corn. Under ordinary room conditions, the seeds remained viable with the germination percentage above 80 for 6, 8, and 10 months for Sweet Corn, Laguna popcorn, and Philippine Hybrid No. 1, respectively (table 2 and fig. 3). As in rice, deterioration

TABLE 2.—Mean germination percentage of three corn types under different storage conditions

DATE	SEED ROOM			COLD STORAGE		
	Sweet Corn	Philippine Hybrid I	Laguna Popcorn	Sweet Corn	Philippine Hybrid I	Laguna Popcorn
<i>1957</i>						
April	100.0	—	—	100.0	—	—
June	94.0	—	—	98.0	—	—
August	92.5	—	—	97.5	—	—
September	85.0	—	—	—	—	—
October	76.5	98.5	—	—	97.8	—
November 7	69.0	—	—	—	—	—
November 16	44.0	—	—	—	—	—
November 30	24.0	—	—	—	—	—
December 16	11.5	96.0	—	95.3	99.0	—
<i>1958</i>						
January 2	2.5	—	—	—	—	—
January 17	3.0	—	—	—	—	—
February 1	1.2	—	—	94.6	—	—
February 16	2.0	91.0	99.5	—	97.0	98.8
March 3	2.0	—	—	—	—	—
March 18	0.0	—	—	—	—	—
April 2	0	—	—	96.0	—	—
April 17	—	85.2	99.8	—	94.2	100.0
May	—	85.8	—	—	—	—
June	—	84.0	97.5	98.7	96.5	100.0
July	—	84.5	—	—	—	—
August	—	64.5	94.2	89.5	94.0	99.2
September 1	—	56.0	—	—	—	—
September 16	—	54.0	—	—	—	—
October 1	—	43.0	—	90.5	—	—
October 16	—	30.5	79.2	—	94.0	96.5
November 1	—	22.5	—	—	—	—
November 10	—	15.2	62.8	—	—	—
November 20	—	10.5	—	—	—	—
November 28	—	5.5	—	—	—	—
December 5	—	3.8	52.8	92.0	—	96.2
December 20	—	2.0	—	—	94.2	—
December 28	—	0	31.0	—	—	—

TABLE 2 (continued)

DATE	SEED ROOM			COLD STORAGE		
	Sweet Corn	Philippine Hybrid I	Laguna Popcorn	Sweet Corn	Philippine Hybrid I	Laguna Popcorn
<i>1959</i>						
January 6	—	—	16.8	—	—	—
January 12	—	—	10.5	—	—	96.0
January 19	—	—	7.5	—	—	—
January 26	—	—	4.2	—	—	—
February 3	—	—	2.2	94.0	—	—
February 10	—	—	0	—	—	—
March	—	—	—	95.0	94.8	95.5
May	—	—	—	94.0	95.2	96.0
July	—	—	—	—	94.0	98.2
September	—	—	—	—	94.8	96.5
November	—	—	—	—	97.2	97.8
<i>1960</i>						
March	—	—	—	—	96.8	96.5
May	—	—	—	—	97.0	97.5
August	—	—	—	—	97.0	98.7

was also very rapid after the critical period (when germination has decreased to 80 per cent). Hawaii Yellow Sweet Corn gave no germination percentage on the 12th, Laguna popcorn on the 13th, and Philippine Hybrid No. 1 on the 15th month.

Philippine Hybrid No. 1 maintained the longest high germinating capacity and Hawaii Yellow Sweet Corn, the shortest. The difference may be due to conditions under which they were subjected. These corn types were placed in storage at different parts of the year because they were not harvested at the same season. Perhaps, Hawaii Yellow Sweet Corn was stored during a part of the year favorable to deterioration while Philippine Hybrid No. 1 was not; germination started October and April, respectively. It is also likely that there are differences when the viability of these various types of corn is critical.

On the other hand, the seeds kept in cold storage remained highly viable (table 2 and fig. 3). Philippine Hybrid No. 1 and Laguna popcorn gave a mean germination percentage of about 96.0 at the latest viability test (36th and 32nd month of storage, respectively). The viability test for Hawaii sweet corn was conducted only up to the 25th month because the remaining seeds had been lost.

The prolonged viability could be due to reduced metabolism of the seed under low temperature and relative humidity, hence longer life span. These conditions apparently prolonged the dormancy period of the seeds. Perhaps the growth of the embryo was arrested as metabolic processes were slowed down by low temperature and humidity.

Within the same crop, the varietal differences in ability to remain viable may be explained by the differential initial viability and inherited qualities of the varieties. William Foster (1946) stated that differential initial vitality is affected by maturity of the seeds; weather conditions during ripening, harvesting and curing; disease or insect injury; and handling of seed prior to storage. He observed that seeds of high initial vitality retain their viability longer under unfavorable conditions than those of lower vitality and that seeds of poor initial vitality may maintain germinative power fairly well under favorable storage but deteriorate very rapidly under poor storage.

Likewise, seeds of different crops like rice and corn retain viability differently, because these crops are genetically, physiologically, and chemically different.

SUMMARY AND RECOMMENDATION

The viability of rice and corn seeds is markedly prolonged by relatively low temperature (59°F) and relative humidity (59.5 per cent). While none of the seeds stored under ordinary seed room conditions germinated after the 20th month, those in cold storage still gave almost 100 per cent germination even after three years.

Dormancy of rice seemed to be prolonged by low temperature and humidity. The assumption needs further verification by using more dormant varieties. Different varieties of rice and corn seem to have different critical viability levels and ability to remain viable. Deterioration of the seeds was very rapid after the critical period. When kept under ordinary seed room conditions, Azucena, S.K. 36 Str. 482, and Peta completely lost their viability on the 16th, 17th, and 20th month of storage, respectively; Yellow Sweet Corn, Laguna Popcorn, and Philippine Hybrid No. 1 on the 13th, 14th, and 16th month, respectively.

It is recommended that seeds of rice and corn be stored in a cold storage room to prolong their viability.

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THE ELECTRICAL RESISTANCE METHOD OF MEASURING SOIL MOISTURE DEPLETION UNDER A CORN CROP¹

A. A. BRIONES and J. G. DAVIDE²

WITH THREE TEXT FIGURES

Plaster-of-paris blocks were made and installed at various depths in a corn field receiving different rates of nitrogen. During resistance periods of dry weather, high electrical resistance of the blocks were obtained indicating low moisture content or high moisture stress. Soil moisture depletion was much greater at the first 6-inch layer in plots receiving nitrogen fertilizer than in those with no fertilizer addition. The addition of nitrogen greatly increased growth and the plants were able to utilize moisture in the lower layer once a greater fraction of the available moisture in the surface layer was used up. In the fertilized plots, moisture depletion during dry periods extended even up to the 18-inch layer.

Moisture adjustments after a rain followed a certain pattern. It took two to three days after a rain before the soil moisture in the lower depths was adjusted to a new level. Plant roots in the lower layer did not seem to immediately utilize the water added by rain.

The lack of adequate moisture in the soil is one of the major factors limiting corn production. Dumenil (1950) reported that nitrogen was second only to moisture as the most important factor limiting corn yields. Nelson (1956) observed that farmers adopted such practices as fertilization, adjustment of plant populations, and tilling of the soil, in order to make best use of the existing soil moisture. There were times, however, when the use of fertilizers as a means of increasing the efficiency of a limited supply of soil moisture brought about disappointing results. In areas where subsoil moistures were depleted and where rainfall was extremely limited, moisture was so deficient that yields did not differ in spite of differing fertility levels.

The utilization and efficiency of applied fertilizers is greatly influenced by the climatic conditions during the season. In the Philippines, corn is grown in both wet and dry seasons. Significantly higher yields and greater responses have been observed more

¹ Central Experiment Station Contribution No. 2058.

² The authors gratefully acknowledge the advice of Dr. Lyle E. Nelson, former visiting professor of Soils.

frequently during the wet season (Tilo, 1957). Lack of moisture supply would probably explain the low efficiency of nitrogen and consequently, the low yield levels during the dry season. Robins and Domingo (1953) found that soil moisture depletion throughout the root absorption zone to the wilting point for periods of 1 to 2 days during tasseling reduced yields by 22 per cent, and for 6 to 8 days, by 50 per cent. They attributed these reductions partly to reduced ear size and partly to reduced fertilization from non-receptive silks.

Russell, *et al.* (1940) conducted a field study of corn and soil moisture relationships. They followed the moisture conditions by installing tensiometers in the field. The data that they found indicated that the zone of moisture absorption began at a shallow depth directly beneath the corn plant and spread laterally until the major portion of the available moisture at that depth has been used. The plants then began utilizing moisture from the soil at a lower depth. At this depth, the zone of moisture absorption once more expanded laterally from a point directly beneath the corn plants until the available moisture was also exhausted. The lateral expansion of the absorption zone occurred at successively lower depths as the growing season progressed.

Shubeck and Caldwell (1955) reported that the amount of moisture in the soil was significantly affected by plant population or density of stand. There was greater moisture depletion in places with thicker than with thinner population density.

Because of the interdependence of the factors associated with crop growth and yield, information on moisture behavior in soils under an existing crop is valuable in interpreting fertility experiments. The investigation reported in this paper was designed to determine the soil moisture fluctuations during the growth cycle of corn, and to relate fertility levels to soil moisture depletion by a corn crop. To accomplish these aims, a method which enables a continuous measurement of the soil moisture conditions in the field throughout the growing season of the crop was selected.

MATERIALS AND METHODS

The plaster-of-paris electrical resistance method (Bouyoucos and Mick, 1940; Bouyoucos, 1954) was used to follow the moisture changes in the field. This method was preferred to the classical gravimetric method because it affords a continuous measurement of the soil moisture at several depths without disturbing the soil

conditions after the original installations have been made. Furthermore, its accuracy is not affected by textural differences. The method as described by Bouyoucos makes use of two units: a small porous absorbent block, and a modified Wheatstone bridge. Two parallel electrodes with leads are cast in the block. When buried in the soil, the block becomes a part of the soil, absorbing moisture from the soil and giving it up readily to the soil so that its moisture content tends to stay in constant equilibrium with the moisture content of the soil. The electrical resistance of the block changes with its moisture content which in turn varies with the moisture content of the soil in which it is buried (Bouyoucos, 1954).

The gypsum blocks used in these studies were prepared in the laboratory of the Department of Soils. After the blocks were made, they were saturated with distilled water. Only those blocks giving a resistance of 400-600 ohms were selected for field installation.

In order to correlate the electrical resistance to soil moisture content, calibration of the blocks were made on composite samples collected at four soil depths in the experimental area. Two soil moisture constants, the wilting percentage and moisture equivalent, were also determined from the same samples.

The field wherein this study was conducted was a Lipa clay loam soil, which was then being used in the corn fertility experiment of the Soils department. The fertilizer treatments consisted of different rates of nitrogen replicated six times. One-third of the total nitrogen required was applied at planting (May 28, 1956) and the remainder was side dressed four weeks later. Two sites were selected on each plot and holes were made. The gypsum blocks were presaturated with distilled water before installation. They were laid horizontally at the 6-, 12-, 18-, and 24-inch depths. After the blocks were installed, the holes were covered with soil, which was tightly packed to bring it near its original bulk density.

The electrical resistance of the block was measured daily for a period of one week starting June 10, 1956. Thereafter up to harvest time (August 28, 1956), readings were taken at three-day intervals. However, if it rained during a scheduled day, readings were taken a day after.

RESULTS AND DISCUSSION

Calibration curves. Table 1 summarizes the electrical resistance of the blocks and the corresponding moisture content of each soil

TABLE 1.—*Electrical resistance and the corresponding moisture content of the different soil layers used in the calibration*

0-6 INCHES		6-12 INCHES		12-18 INCHES		18-24 INCHES	
Electrical resistance	Moisture content	Electrical resistance	Moisture content	Electrical resistance	Moisture content	Electrical resistance	Moisture content
ohms	per cent	ohms	per cent	ohms	per cent	ohms	per cent
440	51.44	450	54.55	450	59.74	400	62.52
440	45.92	450	48.76	450	53.98	440	56.60
440	40.68	450	43.72	450	48.55	440	53.41
480	37.74	480	41.88	450	44.78	460	48.79
500	33.84	540	35.96	500	38.38	660	41.37
940	27.55	940	30.01	700	32.30	1420	35.32
4500	24.34	2900	26.86	1900	30.64	3350	34.01
16000	22.89	8000	26.78	5300	29.64	16100	32.22
81000	20.85	43500	24.09	27500	27.92	69000	31.71
100000	18.21	100000	22.60	94000	26.86	100000	30.05

layer. It will be noted that in the same resistance level, the moisture content differed with soil depth. The relationship between the electrical resistance and soil moisture conditions is best illustrated in figure 1. While there are variations in the magnitudes of these two values with the kind of soil sample used, the curve patterns are very similar. In each curve, the permanent wilting point and moisture equivalent are indicated. The difference between the values of these two soil moisture constants represents the available soil moisture. The electrical resistance method actually measures moisture tension in the soil (Bouyoucos, 1954). Above the moisture equivalent, the method is rather not sensitive. A large variation in moisture content at this point was not reflected in wide changes in the resistance reading. However, below the moisture equivalent level to the wilting point, slight changes in moisture content reflected wide changes in the electrical resistance. On the basis of the free energy concept, Bouyoucos (1954) pointed out that soils at any given electrical resistance level hold their water with the same force or tension. For instance, at 10,000 ohms resistance (fig. 1) soils with 0-6" depth and 18-24" depth hold their respective water with the same force or tension. In other words, these soils have the same degree of wetness even though the former contains 23 per cent moisture and the latter, 32 per cent. The method, therefore, is somewhat ideal for following the available moisture changes in the field. These changes could then be related to moisture depletion by growing crops or adjusting the soil moisture after a rainfall.

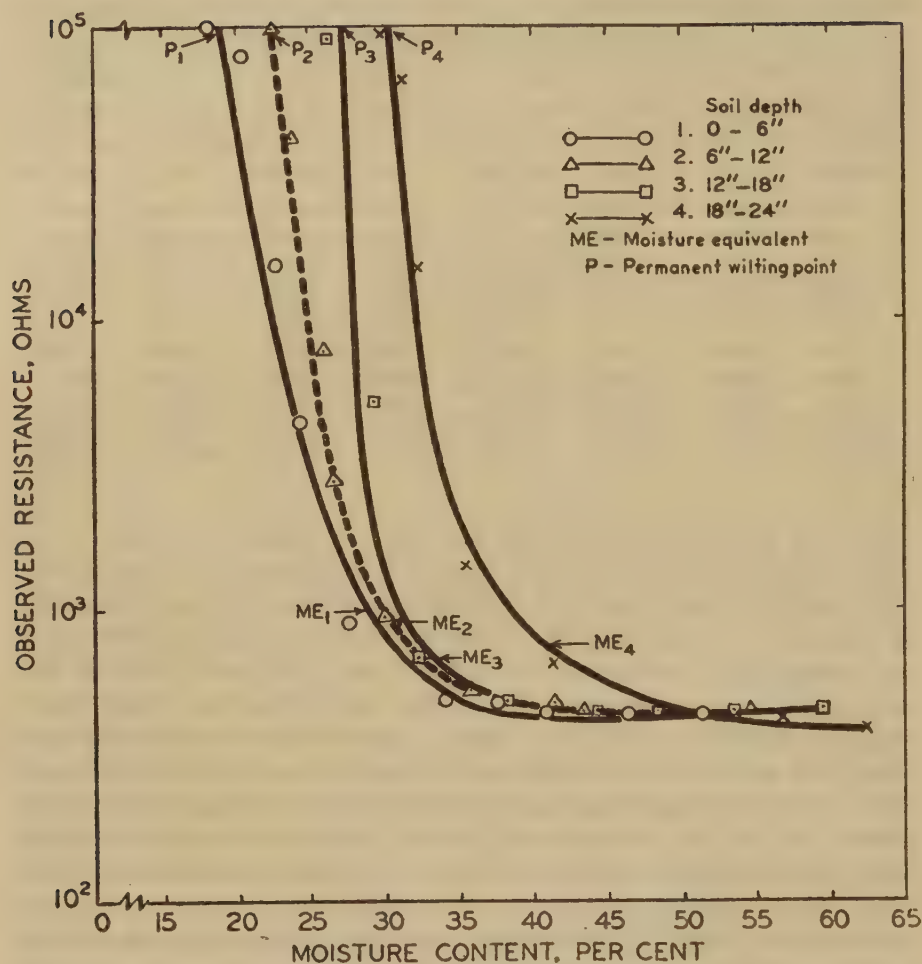


Fig. 1. The relationship between block resistance and moisture content of soils at varying depths.

Moisture changes in the field. The weather during the experimental period was characterized by little or no rain from June 10 to 26, followed by a rainy period from June 27 to July 21, by another dry period from July 22 to 28, and finally ending with a wet period. The rainfall distribution is presented in figure 2c.

Figures 2 and 3 show the changes in the electrical resistance during the growing seasons. It is evident that the fluctuations in the resistance readings were influenced by weather conditions and the kind of crop growth. Readings were higher during dry con-

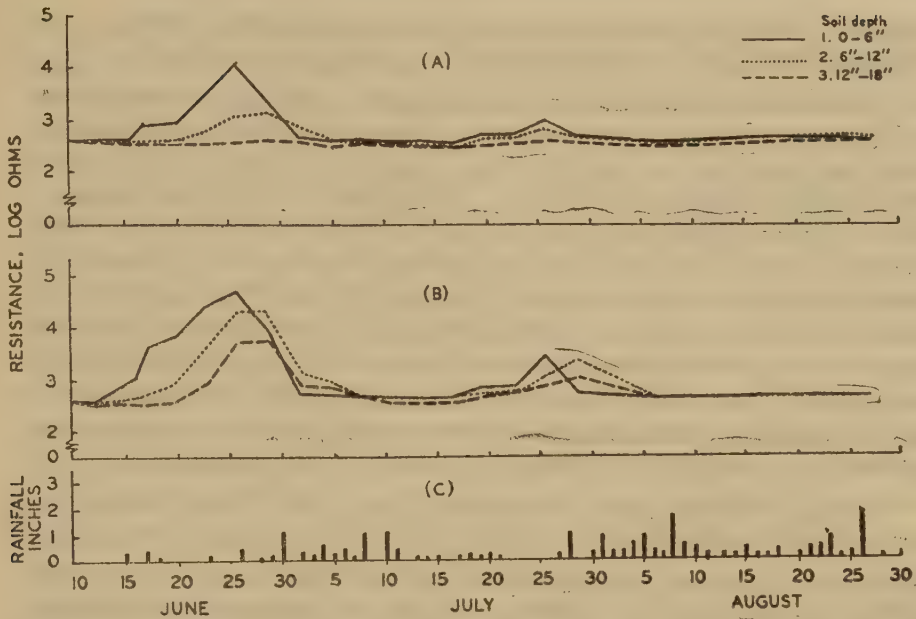


Fig. 2. Observed resistance at different soil depths under a corn crop with various nitrogen levels, (A) ON, (B) 45N and (C) Rainfall distribution during the season.

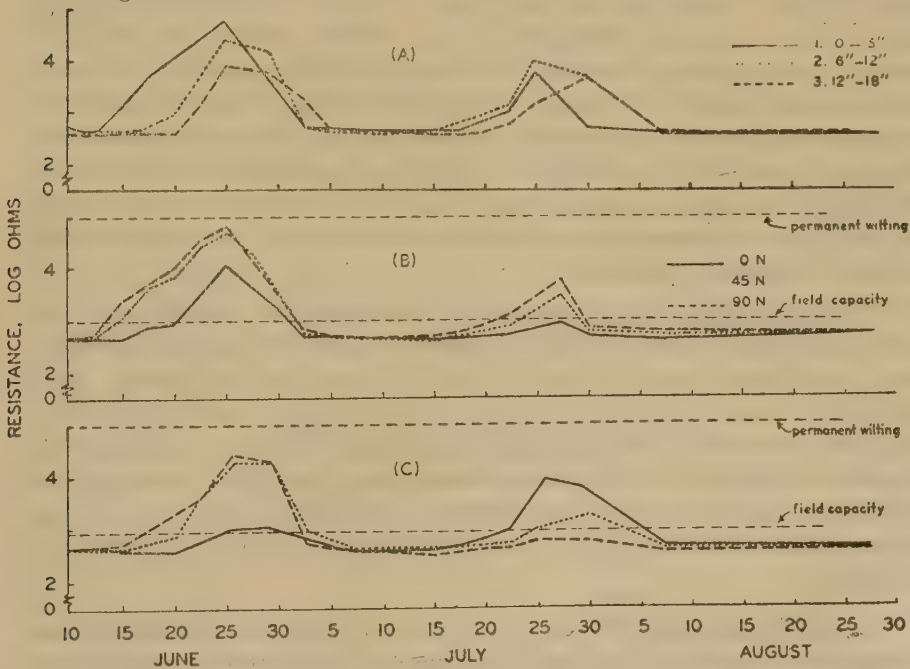


Fig. 3. Observed resistance at different soil depths and at various nitrogen levels, (A) with 90N, (B) at 0-6" with 0, 45, and 90N, (C) at 6-12" with 0, 45, and 90N.

ditions. Moisture depletions were much greater and faster in the upper six-inch layer where most of the roots were confined.

The electrical resistances of the blocks installed in plots without nitrogen application are shown in figure 2A. The highest resistance was recorded on June 26. This could be interpreted as the lowest moisture content in the soil. Alternately, it may also mean a high moisture depletion from the soil by the growing plant. The resistance curves also reveal that during a dry spell, the plant can make use of the water stored in the subsoil layers. This can be seen from the curve of the 6- to 12-inch layer. At the 12- to 18-inch layer, the resistances were essentially constant throughout the growing season, thus indicating no moisture removal by the plants. It could be inferred that in spite of a dry period, there was still water available for the plant in the lower portion of the profile.

A closer examination of the resistances observed following a dry period suggests that two things may have occurred. First, the root absorbing zones were confined in the 0-6 inch layer; once this layer was depleted of soil moisture, there was movement of moisture by capillarity from the lower to the upper layers. Because of the lower moisture content of the surface layers compared with the layer below, a moisture gradient was developed, causing the moisture to move upward. However, Baver (1956) pointed out that the water movement may be slow after the moisture content is reduced to about field capacity. Second, or alternately, the actual root absorbing zone may have extended to and beyond the 6- to 12-inch layer. This was especially true in plots receiving nitrogen treatments where the better vegetative growth was observed. Because of the effect of fertilizers, extensive root systems were necessary to support the increased vegetative growth.

In plots where 45 kilograms of nitrogen per hectare was applied, moisture removal occurred earlier during the dry period and at a faster rate than in plots where no fertilizer was added. Besides, the moisture depletion extended into the lower soil layers. This is evidenced by the electrical resistance curves shown in figure 2B. Greater resistance values were also observed in plots receiving 90 kg. N/ha. At this rate of fertilization, the conditions in the 12- to 18-inch layer were even drier than the same layer in plots with only 45 kg. N.

In all treatments, the depletion of soil moisture was much less during the second dry period. During the last three weeks of the

crop season, the moisture contents of the soil were almost above field capacity. This was due to more rain during the period. In addition, the plants were already maturing and the water requirements were not as great as when the plants were at their vegetative stage.

It is interesting to note that soil moisture adjustments after a rainfall were not uniform throughout the soil profile. Comparison of the curve patterns at various depths (fig. 2A, 2B, and 3A) reveals that after it rained, the 0- to 6-inch layer was the first to be saturated as indicated by a drastic drop in the electrical resistance. It would take two to three days before the underlying layers would attain the same resistance value as the surface layer. The rate of moisture adjustments in the lower layers would depend upon other soil factors controlling the rate of percolation. The observation implies that roots of plants in the lower soil layer depleted of moisture cannot immediately utilize the water added through rainfall.

The relative soil moisture depletion as influenced by crop growth is presented in figures 3B and 3C. Only the resistances of the 0- to 6-inch layer are given. The results obtained in the deeper layers followed a similar trend although the magnitudes were rather lower.

In the upper 12-inch layer, the observed resistances in the various plots were in the order of $90N > 45N > 0$. This showed that moisture depletions were greater in plots receiving 90 kg. nitrogen per hectare than those receiving 45 or none at all. These observations clearly illustrate the high water requirement of a fertilized corn. Because of extensive root system and deeper rooting zone, moisture removal under a fertilized corn was much faster and occurred earlier in a period of dry weather. The grain and stover yields in each treatment are given in table 2. While there were

TABLE 2.—*Grain and stover yields in plots under observation*

NITROGEN TREATMENT	STOVER	GRAIN
<i>kg./ha.</i>	<i>cav./ha.</i>	<i>kg./ha.</i>
0	17.5	898
45	42.4	1330
90	51.4	1573

significant differences attributed to nitrogen fertilization, the yield levels especially in the fertilized plots were rather low. The reason for such low levels may be due to inadequate moisture supply during the early stages of growth. The second dressing of nitrogen was applied four weeks after planting which coincided with the time when there was not enough soil moisture. The fertilizer may not have exhibited its full efficiency.

During the driest period (June 25), the moisture content in the soil in plots treated with 90 kg. nitrogen was close to wilting point. However, the plots receiving no nitrogen still had a good supply of available moisture. Calculations showed that the moisture left in the soil at the 0- to 6-inch layer for plant use during the driest period were in the order of 0.04, 0.15, and 0.28 inch of water in the 90, 45, and 0 nitrogen treatments, respectively.

SUMMARY AND CONCLUSION

The depletion of soil moisture under a corn crop receiving various levels of nitrogen was followed throughout the growing season. The moisture present in the soil at any one time was determined by getting the electrical resistance of plaster-of-paris blocks which were buried at various soil depths. The electrical resistance was previously calibrated with the moisture content of the soil.

The electrical resistance pattern during the entire season followed closely the rainfall distribution. High resistance values which meant low moisture content and greater moisture stress were observed during the dry periods. Moisture depletion occurred earlier in the first 6-inch layer. Once this layer was depleted of moisture, plants utilized the moisture in the next layer.

In the fertilized plots, there was a marked moisture depletion even in the 18-inch layer. As the rate of nitrogen increased, greater moisture depletion occurred as a result of increased plant growth and deeper root penetration.

After it rained, the resistance readings of the surface layers dropped rapidly. However, it usually took two to three days before a marked drop in resistance could be observed at the lower layers. This means that the rate of moisture adjustments at the lower layers is dependent upon the rate of percolation.

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A STUDY OF TWO PROCEDURES IN TRAINING BOARS FOR SEMEN COLLECTION ¹

L. L. CLAMHOY, J. P. ABILAY, and O. A. PALAD

WITH TWO TEXT FIGURES

Fourteen boars trained for semen collection by using the improvised Japanese-type artificial vagina were divided into two groups, according to the two methods of training them: the natural-dummy method and the direct-dummy method. The training of the boars in the first method required twice as much time and effort as that in the second method. Significant differences were found in the duration of the first three successful ejaculations and in the volume of semen collected from them. The difference between the initial motility readings of the two treatments was insignificant.

Semen collection is an important phase of work in artificial insemination. Good quality semen can only be collected from animals trained to serve an artificial vagina. In training boars, certain procedures have to be regularly followed. These procedures have never been standardized because boars have different breeding individualities and preferences.

This study was done in the Department of Animal Husbandry, U. P. College of Agriculture, from January, 1959 to March, 1960, to find out the best possible procedures that will train boars to serve an artificial vagina in the shortest time possible and at different circumstances.

Frank (1952)² described the procedure in collecting semen from boars with the use of the Missouri Type artificial vagina. Before collecting, he said, the inner surface of the tube is lubricated and the entire apparatus is immersed in water heated to about 46°C. T teaser sow is used. The sow is placed in a simple stanchion and tied to a wall by means of a rope tied around his upper jaw. When the boar mounts and attempts to copulate, the open end of the tube is placed in front of his sheath so that the penis could pass into the

¹ Central Experiment Station Contribution No. 2059. Research Project No. 1402.

² A. H. Frank, "Artificial Insemination in Livestock Breeding," United States Dept. Agric. Cir. No. 567 (April, 1952).

tube. As his penis enters the tube it is manipulated with a pulsating motion by the attendant's hand, thus encouraging continued copulation and ejaculation.

Frank also stated that sexual attraction plays a relatively small part in the mating behavior of a boar, so that it is usually not necessary to have a sow in heat. Boars may likewise be readily stimulated to mount a dummy sow.

Niwa (1958)³ described semen collection with the aid of a wood or metal dummy. He said that the size of the dummy should fit the size of the boar so that it could ride with ease. The dummy has legs placed wide apart to avoid tumbling over, and is padded with tanned pig skin or soft cloth. It is first smeared with the sow's urine or secretion from the vagina. The boar is then led to it. The collector usually stays on the right side of the dummy, holding the artificial vagina by his right hand and the sheath by his left hand, and puts the end of the penis into the artificial vagina. When the end of the penis fits into the narrow part of the artificial vagina, the collector slowly grasps the cylindrical part of the penis with the thumb, index finger and middle finger of his right hand, and subjects it to strong pressure. The penis gets longer and starts to ejaculate. The collector holds the semen container with his left hand, and continues pressing the end of the penis throughout the ejaculation with his right hand.

MATERIALS AND METHODS

In this study, 14 boars of the Berkshire, Berkjala, Duroc-Jersey and Berkshire-Berkjala crosses were used. They ranged in age from 7 to 11 months. The boars were divided into two groups, seven to each side, and assigned at random to the two methods of training: the natural-dummy method, and the direct-dummy method.

In the natural dummy method, each boar was allowed to serve in heat sows once or twice before training him to use the dummy. In the direct-dummy method, the boars were not allowed to serve in heat sows in natural breeding prior to and during the training period.

The method of semen collection used was patterned after that described by Niwa (1958), which used the improvised Japanese-

³ Tazaemos Niwa, "Artificial Insemination with Swine in Japan," Nat. Inst. Agric. Sci., Chiba City, Japan (August, 1958).

⁴ *Ibid.*

type artificial vagina (fig. 1). The collector stayed either at the left or at the right side of the mounted boar, with the hand near

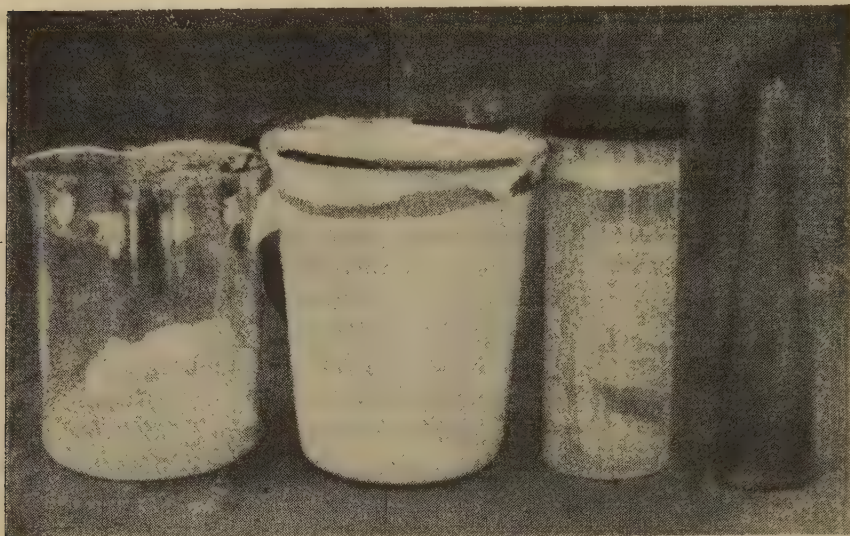


Fig. 1.—From left: a beaker with gelatinous portion of boar semen, a calibrated porcelain pitcher with gauze held by a rubber band, a capped plastic bottle with the liquid portion of boar semen, and an improvised Japanese-type artificial vaginae for collecting semen from boars.

the boar holding the artificial vagina and the hand far from the boar holding the semen container (fig. 2).

In training the boars, the following requirements had to be met: (1) the boars should be in good physical condition and at least seven months of age, (2) only one operator should do the entire job of training the animal, (3) training should always start and end at the pen where the boar is confined, (4) training should be done punctually at the same hour, and (5) the operator should have absolute patience, kindness, understanding, and love for the animal.

At the designated hour of each day, the collector or trainer took the dummy with him to the pen of the boar. At first the boar was afraid of the dummy and ran or shied away from it. This procedure was repeated for 30 minutes every day until the boar became acquainted with the operator and the dummy. Later on the boar would approach the dummy, smell it, go around it, and playfully bite it and push it with his snout, often toppling it. Then he



Fig. 2.—Collecting semen from boars trained to use a four-legged dummy made of lumber and heavily padded with gunny sack as cushion.

attempted to mount. The trainer held the dummy in place at the opposite end where the boar was and maneuvered it so that it was always in front of the boar. In about two weeks' time the boar had learned to trust the trainer. He allowed himself to be handled at the sheath. At this time, he was also stimulated to protrude his penis. The collection of semen was done with the artificial vagina.

RESULTS AND DISCUSSION

Length of training period. From table 1 it can be seen that the boars which were first allowed to serve in heat sows before they were trained to use the dummy (natural-dummy method) required a much longer training period, varying from five to 28 days, or an average of 13.6 days.

The boars trained to mount the dummy without the benefit of natural breeding (direct-dummy method) required a much shorter training period, varying from five days for almost all of the boars to 14 days for one boar, or an average of 7.1 days.

TABLE 1. Breed, age, and length of time required in training boars, and duration, volume, and initial motility of the first three successful ejaculations

NATURAL-DUMMY METHOD														
H.N.	BREED	AGE AT TRAINING DAYS OF (mos.) TRAINING	DURATION OF EJACULATION (min.)				VOLUME (cc.)				PER CENT INITIAL MOTILITY			
			1	2	3	Ave.	1	2	3	Ave.	1	2	3	Ave.
545	BJ	11	18	6	3	4	4.3	100	30	90	66.6	50	50	50
619	BJ × BS	7	14	4	5	8	5.6	45	25	80	50.0	70	70	70
"0"	BS	10	28	5	7	4	5.3	75	140	50	88.3	70	70	70
542	BS	7	11	8	8	12	9.3	100	100	125	108.3	60	60	60
7	DJ	8	12	4	6	3	4.3	75	120	50	81.6	70	70	70
9	DJ	7	7	6	5	8	6.3	115	70	100	95.0	60	60	60
11	DJ	7	5	5	8	10	7.6	100	150	120	123.3	60	60	60
Total		57	95	38	42	49	42.7	610	635	613	613.1	440	440	440
Average		8.4	13.6	5.4	6.0	7.0	6.1	87.1	90.7	98.9	87.6	62.8	62.8	62.8
DIRECT-DUMMY METHOD														
587	BS × BJ	7	5	2	5	8	5.0	6	75	110	63.6	50	50	50
544	BS	7	14	6	8	8	7.3	100	100	100	100.0	60	60	60
8	DJ	7	5	11	8	11	10.0	100	110	100	103.3	60	60	60
18	DJ	7	5	10	6	8	8.0	100	75	90	88.3	70	70	70
35	DJ	7	9	8	5	10	7.6	75	60	100	78.3	70	70	70
10	DJ	7	5	18	8	6	7.3	110	50	75	78.3	60	60	60
36	DJ	7	7	5	4	6	5.0	50	50	75	58.3	50	50	50
Total		49	50	50	44	57	50.2	541	520	650	572.1	420	420	420
Average		7	7.1	7.1	6.2	8.1	7.1	77.2	74.2	92.8	81.7	60	60	60

It appears that boars allowed to serve natural breeding first before making them use the dummy needed twice as much time and effort as those which used the direct-dummy method. This difference could be due to the experience gained by the former while serving in heat sows. The difference was significant.

Duration of the first three successful collections. The average length of time of ejaculation by boars trained under the natural-dummy method varied in the three collections: 5.4 minutes for the first successful collection, 6 minutes for the second successful collection, and 7 minutes for the third successful collection, or an average of 6.1 minutes for the three collections.

For the direct-dummy method, the time required was 7.1, 6.2, and 8.1, respectively, or an average of 7.1 minutes for the three successful collections. The difference was significant at 5 per cent level.

The boars trained without benefit of natural mating had a relatively longer ejaculation time. However, there was a tendency to lengthen the time of ejaculation when the boars became trained for semen collection. This could be due to their acquaintance with the treatment given them and the confidence they acquired from the collector or trainer.

Volume of semen of the first three successful collections. The average amount of semen collected under the natural-dummy method varied from 87.1 cc. for the first ejaculate, 90.7 cc. for the second ejaculate, and 98.9 cc. for the third ejaculate, or an average of 87.6 cc. for all ejaculates from all boars in the first three collections.

From boars trained in the direct-dummy method, the average amount of semen collected varied from 77.2 cc. for the first ejaculate, 74.2 for the second ejaculate, 92.8 for the third ejaculate, or an average of 81.7 cc. for all ejaculates in the first three collections.

The difference in volume between the two treatments was significant at 5 per cent level.

It appears that the amount of semen collected from the boars which served in natural breeding prior to their training with the dummy was significantly of much bigger volume than that of the boars deprived of the experience in natural breeding. Natural service and past pleasant treatment served as powerful stimuli for larger ejaculates.

Initial motility of semen of the first three successful collections. The initial motility readings for the two groups show that the natural-dummy group had a higher percentage of motile spermatozoa than the direct-dummy group, its average for the three successful collections varying from 50 to 70 per cent, or an overall average of 62.8 per cent. With the latter group, the average reading varied from 50 to 70 per cent or an overall average of 60 per cent. The difference was insignificant. The difference in motility in favor of the natural dummy method group could be due to the fact that the boars were a little bit older and that they had been used in natural breeding.

SUMMARY

1. Fourteen boars of the Berkshire, Duroc-Jersey, Berkjala and Berkshire-Berkjala crosses were divided into two groups according to the method of training intended for them: the natural-dummy method and the direct-dummy method.

2. The boars trained in the direct-dummy method required only 7.1 days to prepare them for the use of the artificial vagina; whereas those in the natural-dummy method required twice as much time.

3. The duration of the first three successful ejaculations, and the volume of semen collected from them were statistically significant at 5 per cent level.

4. The initial motility of the first three successful ejaculates was higher in the natural-dummy method than in the direct-dummy method by 4.2 per cent units, but the difference was statistically insignificant.

THE VALUE OF SOYBEAN OIL MEAL IN RATION FOR GROWING-FATTENING PIGS¹

R. B. PUYAOAN, R. D. PENGSON, AND J. A. EUSEBIO²

WITH TWO TEXT FIGURES

When compared, the fish meal and soybean oil meal-fish meal lots showed no significant differences in feed conversion efficiency and gain in weight. On the average, Lot I (fish meal) needed 4.71 kilograms and Lot II (soybean oil meal-fish meal), 3.94 kilograms of feed to produce a kilogram gain in weight. It appeared that soybean oil meal can profitably replace one-half of the fish meal in the College swine-growing ration.

Soybean oil meal is a processed by-product of soybean seeds. It is obtained after extracting oil from the beans. It is cheaper than fish meal and, in the United States and other countries, it has been found to be a good protein supplement in swine rations. An analysis made locally showed that soybean oil meal contains 10.19 per cent moisture, 36.40 per cent crude protein, 0.90 per cent crude fat, 6.75 per cent crude fiber, and 4.59 per cent ash. As the contents show, it is relatively low in fat.

Many investigations have shown the favorable effect of soybean oil meal in rations for pigs raised in either confinement or pasture. Carrol *et al.* (1939a) reported that on good pasture, soybean oil meal was the only protein supplement with minerals which gave satisfactory results when fed to swine of different weights and of all ages. Vestal (1945) noted that the pigs on pasture had an increased gain in weight when half of the meat and bone scraps was replaced with soybean oil meal. Becker *et al.* (1947) reported that a mixture of two parts ground oats and one part soybean oil meal gave excellent results when fed to weanlings on brome grass pasture up to market weight. Likewise, Robison (1951) observed that when soybean oil meal was substituted for an equal amount of animal protein from meat scraps or dry-rendered tankage in swine rations, pigs on good pasture ate 8.9 per cent more feed and gained weight 6.4 per cent faster than the animal protein-fed lot.

¹ Central Experiment Station Contribution No. 2060.

² The authors are grateful to Drs. V. Villegas and F. Mensalvas, and Mr. Q. Sugon for their assistance.

On the other hand, Carrol (1940) and Krider *et al.* (1948) reported that the feed conversion efficiency was almost the same when pigs were fed a mixture of corn plus tankage and corn plus soybean oil meal. They also said that on a price basis, soybean oil meal is more profitable than tankage as a source of protein in the ration.

Although soybean oil meal is deficient in methionine as reported by Carrol and Krider (1956), an experiment by Robison (1947) revealed that in practical dry-lot feeding, the addition of methionine in corn and soybean oil meal supplemented with minerals and vitamins failed to bring about an advantage in pig growth.

Evans (1952) compared soybean oil meal and white fish meal as protein supplements in a diet of two parts barley meal, one part fine rice bran, and a small amount of lucerne meal and minerals. He found that the young pigs had the same rate of growth. Becker *et al.* (1954) reported that soybean oil meal was superior to Menhaden fish meal in a ration of 10 per cent protein; however, they were of equal value in a ration containing 18 per cent protein.

Under local conditions, Jauregui (1951) found that pigs fed with soybean oil meal in combination with protein supplement of animal origin (tankage) made faster and more economical gains than those fed with rations without soybean oil meal.

MATERIALS AND METHODS

Six Berkshire and 14 Berkjala weanlings, each approximately two months old, were involved in this study. Two trials, each covering a period of 30 weeks, were conducted. The first trial made use of six Berkshire and four Berkjala weanlings. They were assigned into two lots according to weight, age, and sex. The entire 30-week feeding test was divided into three 10-week feeding periods. Lot I, designated as control, was given the College swine ration with fish meal alone as the main source of protein. Lot II was practically the same as the control lot, except that soybean oil meal protein was substituted for 50 per cent of the protein fish meal in lot I. The composition of the rations given to the two lots during the whole feeding period is given in table 1.

Identical care and management was given to all the pigs in the two lots. All the animals were raised in close confinement and camote soilage was provided daily in-between feeding. The pigs

TABLE 1.—Composition and calculated analyses of growing-fattening ration

INGREDIENTS	FIRST 10-WEEK FEEDING PERIOD		SECOND 10-WEEK FEEDING PERIOD		THIRD 10-WEEK FEEDING PERIOD	
	Lot I	Lot II	Lot I	Lot II	Lot I	Lot II
Ground yellow corn	15	15	20	20	25	25
Fine rice bran	60	60	60	60	60	60
Copra meal	18	18	16	16	13	13
Soybean oil meal	—	4.3	—	3	—	1.5
Fish meal	7	3.5	4	2	2	1
Mineral mixture ^a	1.5	1.5	1.5	1.5	1.5	1.5
Calculated Analyses:						
Protein, per cent	17.40	17.30	15.60	15.66	14.20	14.28
Fat, per cent	2.78	2.53	2.62	2.48	2.48	2.42
Fiber, per cent	12.21	12.46	12.06	12.13	11.81	11.86
Prod. Energy, Cal/kg.	1703.90	1686.36	1742.40	1744.16	1787.50	1784.97

^a Composed of equal parts of ground shells and common table salts.

were weighed weekly from the start of the experiment. The weekly feed consumption, condition, and health of the animals were noted.

The second trial was designed to confirm the results obtained from the first. In it, 10 Berkjala weanlings were assigned to two lots. The rations given to the two groups were the same as those in lots I and II of the first trial. Also, the same observations were noted.

RESULTS AND DISCUSSION

Growth curve. The data for growth curves were taken from the first trial. Figure 1 shows the weight-time growth curves of the pigs in the two lots. There were similar growth patterns in both lots up to the 30-week feeding period. Growth rates in the two lots were very rapid during the third 10-week period. However, the fish meal-soybean oil meal combination was superior to fish meal alone.

Rate of gain. The average daily gain in weight is shown in figure 2. At any period during the experiment, the pigs given fish meal-soybean oil meal combination had a higher gain in weight than those given fish meal alone. This corroborates the findings of Fairbanks *et al.* (1944) which showed that the combination of plant and animal proteins (tankage and fish meal) in a complete ration for weanling pigs on confinement gave the highest gain in weight com-

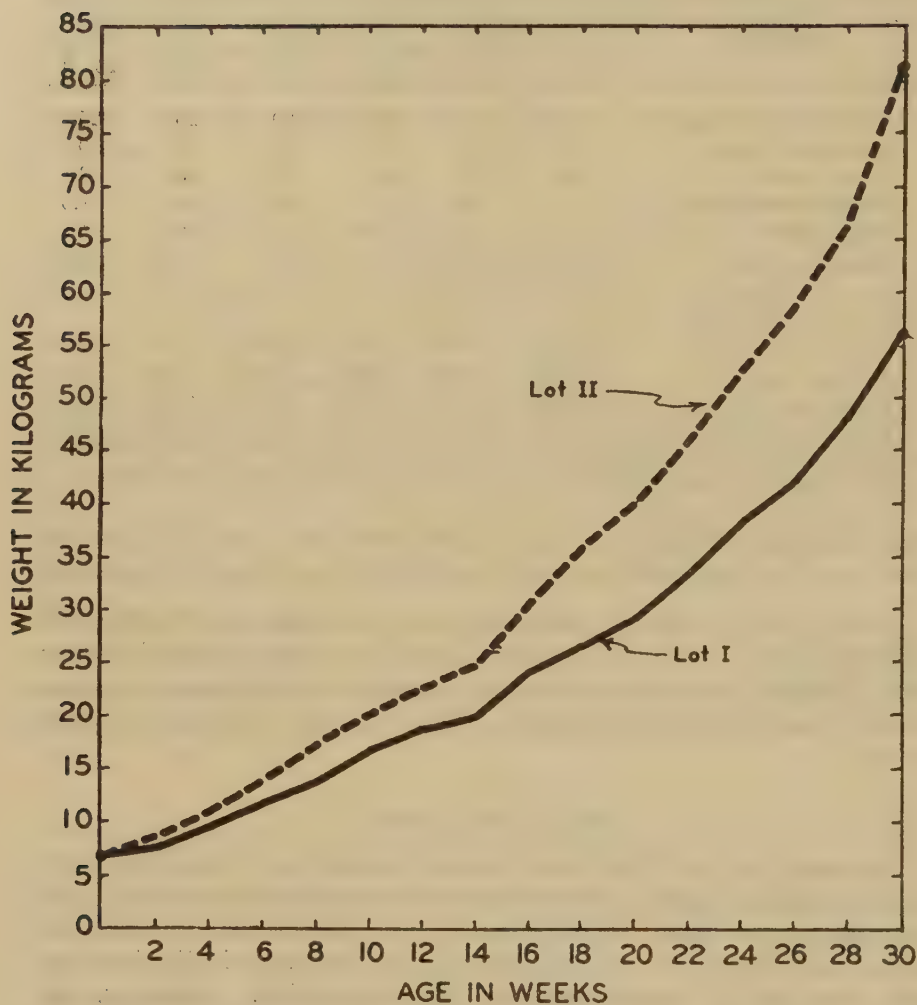


Fig. 1.—Weight-time growth curve for pigs in the two lots.

pared to pigs fed with plant protein alone. The difference in weight gain between the two lots were found to be insignificant.

Feed conversion efficiency. On the average, lot II required only 3.94 kilograms of feed to produce one kilogram gain in weight compared to 4.71 kilograms for lot I (fig. 2). The results agree with Vestal's report of a conversion efficiency of 4.06 with animal protein alone and 3.75 with animal protein combined with plant protein.

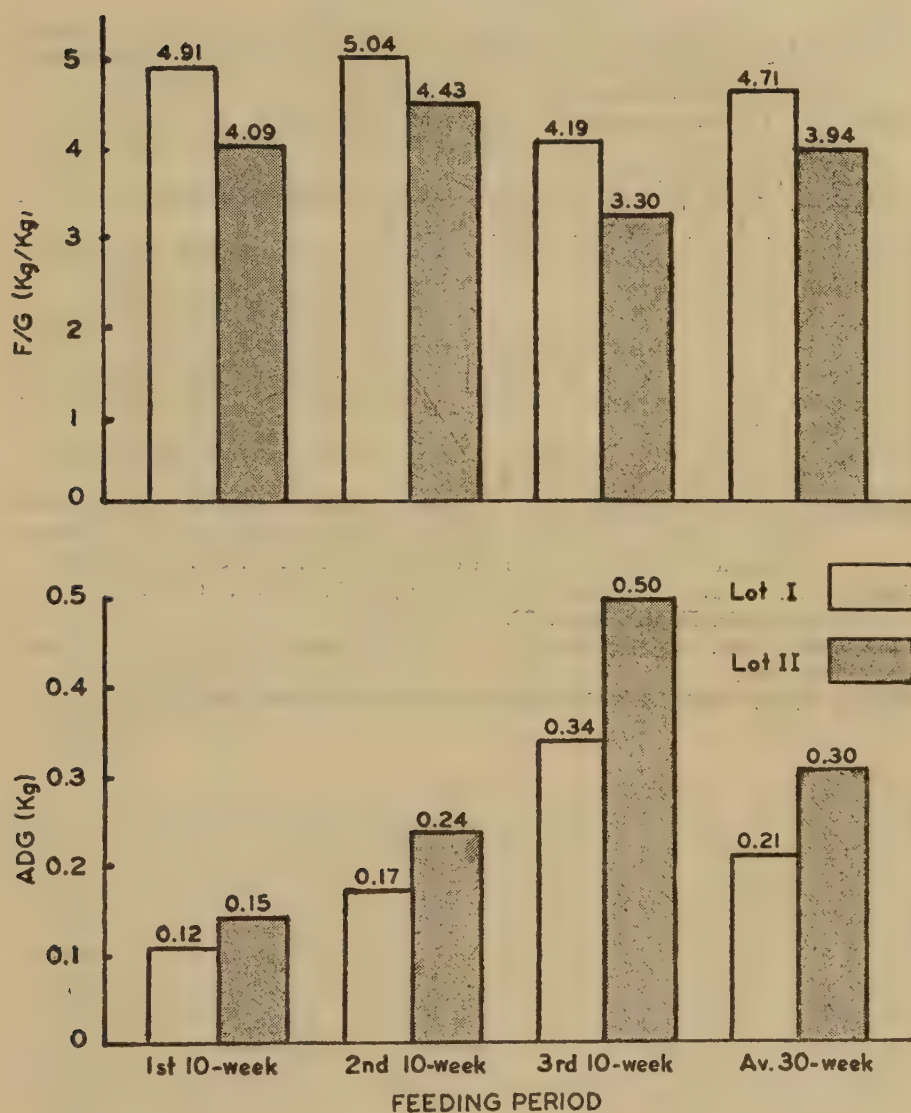


Fig. 2.—Feed conversion efficiency and average daily gain in weight.

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THE EFFECT OF VARIOUS DILUENTS WITH OR WITHOUT ANTIBIOTICS ON STORAGE TIME AND FERTILIZING CAPACITY OF COCK SEMEN ¹

MARIO M. LABADAN and AMADO C. CAMPOS

In general, diluted semen gave a slightly lower fertility than undiluted semen. Of the three diluents tested, Ringer's solution ranked first, Locke's solution second, and skimmilk third in maintaining fertility.

It was apparent that the fertility of semen stored at 10°C. for three hours was significantly higher than that stored at room temperature. Irrespective of the storage temperature and dilution rate used, the fertilizing quality of stored semen decreased as the storage time was increased. In the case of semen diluted with skimmilk stored at room temperature and of semen diluted with Ringer's solution stored at 10°C. for one hour, the fertility ratings obtained were even higher than that of the undiluted semen stored for the same length of time.

The addition of penicillin at the rate of 1000 units per ml. to diluted and undiluted semen proved detrimental. However, fertility was increased with the addition of 500 units of penicillin to semen diluted with either skimmilk or Ringer's solution and stored at 10°C. The addition of penicillin did not show any marked effect on fertility of semen held at room temperature.

Dilution seems to be the most logical approach to an efficient use of cock semen for artificial insemination, inasmuch as semen yield per rooster is small and the number of spermatozoa per millimeter is relatively high. However, poultry insemination work is handicapped by lack of diluents that maintain the high fertilizing quality of pure semen and make possible its storage for a longer period of time. Lengthening the storage time of semen is important because inseminating immediately after collection is not always possible. In transporting fowl semen from one place to another, for instance, some time has to elapse before insemination can be done.

In the Philippines, with the growing popularity of raising layers in cages, the need for inseminating hens artificially is felt. No diluent has as yet been found satisfactory. Therefore, this study attempts to find a suitable diluent to be used in this country. It was

¹ Central Experiment Station Contribution No. 2061.

conducted from September, 1958 to August, 1959, in the Poultry Division, Department of Animal Husbandry.

MATERIALS AND METHODS

Throughout the experiment, semen samples from 15 individually-caged White Leghorn cockerels, given feed and water *ad libitum*, were collected by the abdominal massage method as described by Burrows and Quinn (1935). After thorough mixing, the semen was immediately transferred to a water-bath kept at room temperature (28.7°C.). The pooled semen was then apportioned to the different lots by means of a graduated tuberculin syringe.

The diluents used were Ringer's solution, Locke's solution, and skimmilk. Ringer's and Locke's solutions were prepared in the Nutrition Laboratory of the Department of Animal Husbandry. The fresh supply of skimmilk was obtained from the Dairy Division of the same department, and every batch was heated and maintained at 92°C. in a water bath for 10 minutes, in order to reduce the bacterial count.

Experiment I considered as variables the three diluents, degree of dilution (1:2.5 and 1:5.0), and storage at room temperature and at 10°C. In all cases, the duration of storage was fixed at one hour. Undiluted semen subjected to the same treatment served as control.

Experiment II had the same design as experiment I except for the duration of storage; instead of one hour, semen was held for three hours.

The same variables in experiment II were used in experiment III except for the addition of 500 units of penicillin per ml. of diluted and undiluted semen. In experiment IV, 1000 units of penicillin, instead of 500 was added per ml. of diluted and undiluted semen.

To make the 1:2.5 and 1:5.0 dilutions, pure semen was diluted with the appropriate amount of diluents stored under the same water-bath temperature (28.7°C.), thus preventing temperature shock on the spermatozoa. In experiments III and IV, penicillin was added to the semen after dilution. All samples were kept in 5-ml. vials stoppered with cotton and held in a water-bath before transfer to storage temperatures.

The initial motility of sperms was determined under the high power of the microscope immediately after collection and dilution.

Motility was scored from one to five based on a modified rating by Smith, (1949) (table 1). Final motility was likewise scored after the prescribed storage period.

TABLE 1.—*Index of motility rating*

INDEX OF MOTILITY	RATE OF MOVEMENT
5	Vigorous progressive motion with swirling.
4	Progressive motion of the majority, but no swirling.
3	Majority motile, less than half progressing actively.
2	Majority stationary with vibrating tails. Few or none actively progressing.
1	Only a few showing any movement.

The average of three counts on the Spencer Bright-Line haemocytometer was used as an estimate of sperm density after making each collection.

A total of 150 White Leghorn pullets, not previously inseminated or mated, and confined in individual wire cages, were used in the fertility test.

By means of a tuberculin syringe, each pullet was inseminated weekly with 0.1 ml. of either pure or diluted semen. Inseminations were restricted in the afternoon after most of the pullets have laid.

In a preliminary study, the pullets assigned to all the lots in each experiment took approximately one hour to inseminate. Hence, the time interval in the insemination of the first and last birds in each experiment was about one hour. This difference in time of insemination caused differences in storage time of semen for the various lots. To shorten the insemination time to within the prescribed storage time, the pullets were divided into three groups; each group was inseminated weekly but on different days so that a lesser number of birds was inseminated at a time. Pullets with a hard-shelled egg in the oviduct were not inseminated. Since the pullets were used repeatedly in the series of four experiments, a period of two weeks after the last insemination was made to elapse before another experiment was started. At the beginning of each experiment and before the first insemination was made, two eggs from each bird were incubated in order to ascertain their infertility.

At the start of each experiment, the eggs laid on the day of insemination and the day after were discarded. Thereafter, all eggs

laid by the inseminated birds were collected daily and marked with the dam number and date. The eggs were set weekly on a forced-draft incubator and fertility was determined by candling on the fifth day. Eggs suspected of being infertile after candling were broken and examined macroscopically for evidence of embryonic development before they were finally classified as infertile. Each experiment lasted until at least 100 eggs from each lot had been tested. A total of 6,114 eggs were collected and tested.

RESULTS AND DISCUSSION

Effect of various diluents and dilution rates on fertility. Data in experiment I showed that diluted semen gave a slightly lower fertility than undiluted semen (table 2). Of the three diluents tested, Ringer's solution ranked first (85.7 per cent), Locke's solution second (82.4 per cent), and skimmilk third (80.6 per cent) in maintaining fertility.

TABLE 2.—*Effect of the various diluents and dilution rates on fertility^a*

DILUENT	DILUTION RATE				Combined fertility of 1:2.5 & 1:5.0 dilution
	1:2.5		1:5.0		
	No. of egg set	Per cent fertility	No. of egg set	Per cent fertility	
Ringer's	212	90.0	263	81.4	85.7
Locke's	205	79.5	205	85.4	82.4
Skimmilk	214	88.8	225	72.4	80.6
Average	210	86.1	231	79.7	82.9
(Undiluted semen, 228 eggs set, 88.2 per cent fertility)					

^a Combined data from semen stored at room temperature and 10°C. for 1 hour.

The combined data for the three diluents showed that dilutions of 1:2.5 registered 86.1 per cent fertile eggs, whereas with 1:5.0 dilution, 79.7 per cent. This trend tended to agree with the findings of Gordon and Phillips (1951), and Wilcox (1958), that the fertility rating of semen diluted at 1:2 was generally higher than that at 1:5.0. Rowell and Cooper (1957) suggested that the principal cause of decreased fertility with increased dilution was the decrease in number of live spermatozoa inseminated.

Semen diluted at the rate of 1:2.5 with either Ringer's solution or skimmilk had approximately the same fertilizing capacity as un-

diluted semen. Dilutions at the rate of 1:5.0 gave a consistently lower fertility than undiluted semen. The discrepancy found with Locke's solution could be attributed to a significantly low fertility obtained in one setting in this lot.

Effect of storage temperature and storage time on fertility. Data on the effect of storage temperature and storage time on fertility of diluted and undiluted semen were taken from experiments I and II, using 1:0.0 and 1:2.5 dilutions (table 3). The fertility of semen stored for either one or three hours at 10°C. was, on the average, significantly higher than that stored at room temperature. At room temperature, the average for all diluted samples stored for three hours gave a slightly higher fertility than the undiluted sample. In the case of semen diluted with skimmed milk stored at room temperature and of samples diluted with Ringer's solution stored at 10°C. for one hour, the percentages of fertility obtained were markedly higher than the corresponding fertility rating from the undiluted semen. These findings showed that the full fertilizing capacity of cock semen diluted with appropriate diluents to 1:2.5 could possibly be maintained for shorter periods of storage under either room temperature or 10°C.

The difference between the average fertility of diluted semen stored for one hour at 10°C. and that at room temperature was small (3.6 per cent), whereas after three hours, the difference between the two treatments was great (39.5 per cent). The highest fertility obtained with diluted semen stored for three hours at room temperature was apparently significantly lower than the fertility of either diluted or undiluted semen stored for the same length of time at 10°C.

The average room temperature recorded during the entire experiment was 28.7°C., ranging from 24.5° to 32.5 C. Hunsaker *et al.* (1956) observed that semen at 30°C. immediately lost its fertilizing power partly because of bacterial contamination. Undoubtedly, bacteria developed much more rapidly at 30°C. than at 10°C. The better results obtained in this study with semen stored at 10°C. for three hours might also be attributed to the preserving effect of lowered metabolic rate of sperms.

Under three hours storage at 10°C., dilution with Ringer's solution resulted in very much lower fertility than that with skim milk and Locke's solution. The latter diluents maintained the same level

TABLE 3.—*The effect of storage temperature and storage time on fertility of diluted and undiluted semen* ^a

ROOM TEMPERATURE										10° C.			
1 hour		3 hours		Combined fertility at 1 and 3 hours storage		1 hour		3 hours		Combined fertility at 1 and 3 hours storage			
No. of egg set	Per cent fertility	No. of egg set	Per cent fertility	No. of egg set	Per cent fertility	No. of egg set	Per cent fertility	No. of egg set	Per cent fertility	No. of egg set	Per cent fertility	No. of egg set	Per cent fertility
Undiluted	120	86.7	144	7.6	264	47.2	108	89.8	154	66.2	262	78.0	
Ringer's	105	84.8	105	15.2	210	50.0	107	95.3	121	32.2	228	63.8	
Locke's	105	77.1	120	15.0	225	46.5	100	82.0	139	77.7	239	79.8	
Skimmilk	112	91.1	125	28.0	237	59.6	102	86.3	151	66.9	253	76.6	
Average of diluted	107	84.3	117	19.4	224	51.8	103	87.9	137	58.9	240	73.4	

^a 1:2.5 dilution rate was used. Undiluted semen inseminated immediately after collection = 103 eggs set, 94.2 per cent fertility.

of fertility as undiluted semen. The differences between Locke's and Ringer's solutions is the presence of dextrose in Locke's solution. Skimmilk contains a considerable amount of lactose. In a recent study, Wilcox and Shaffner (1958) found that the addition of fructose to stored semen prior to insemination increased fertility. From these findings it would appear that the presence of sugar in the diluents might have caused beneficial effects on stored semen.

Effect of antibiotic on the preserving quality of stored semen. The effect of penicillin on stored diluted and undiluted semen was determined in experiments II, III, and IV. The addition of 500 units of penicillin per ml. of diluted and undiluted semen stored at 10°C. for three hours resulted in 55.6 per cent fertility. When 1000 units of penicillin was added, fertility was 37.7 per cent, as compared with 48.8 per cent for the control (table 4a). The same

TABLE 4a.—*Effect of two concentrations of penicillin on the preserving quality of semen stored at 10°C. for 3 hours*

PER CENT FERTILE EGGS PRODUCED							
Without penicillin				With penicillin			
				500 units per ml.		1000 units per ml.	
	No. of egg set	Per cent fertility		No. of egg set	Per cent fertility	No. of egg set	Per cent fertility
Ringer's	1:2.5	121	32.2	120	56.7	100	49.0
	1:5.0	124	32.2	101	40.6	114	15.8
Locke's	1:2.5	139	77.7	155	76.1	106	54.6
	1:5.0	137	46.7	123	46.0	102	27.4
Skimmilk	1:2.5	151	66.9	158	76.6	101	39.6
	1:5.0	137	19.7	133	27.8	103	24.3
Undiluted		154	66.2	122	65.6	103	53.4
Average		138	48.8	130	55.6	104	37.7

trend of fertility was observed with semen stored at room temperature for three hours. The addition of 500 units of penicillin to diluted and undiluted semen gave 15.1 per cent fertile eggs, that of 1000 units penicillin, 9.4 per cent, as compared to 14.7 per cent obtained with diluted and undiluted semen without penicillin (table 4b).

Storage at 10°C. for three hours, and the addition of 500 units of penicillin per ml. of semen diluted with either skimmilk or Ringer's solution gave higher fertility ratings than the corresponding lots without penicillin. However, the same treatment of undiluted semen and of semen diluted with Locke's solution showed no

TABLE 4b.—*Effect of two concentrations of penicillin on the preserving quality of semen stored at room temperature for three hours*

		PER CENT FERTILE EGGS PRODUCED					
		Without penicillin		With penicillin			
				500 units per ml.		1000 units per ml.	
		No. of egg set	Per cent fertility	No. of egg set	Per cent fertility	No. of egg set	Per cent fertility
Ringer's	1:2.5	105	15.2	104	16.3	83	2.4
Ringer's	1:5.0	103	15.5	135	10.0	100	5.0
Locke's	1:2.5	120	15.0	121	6.6	87	12.6
Locke's	1:5.0	139	12.2	119	8.5	101	22.7
Skimmilk	1:2.5	125	28.0	100	42.0	88	19.3
Skimmilk	1:5.0	131	7.6	134	10.4	88	1.1
Undiluted		106	9.4	105	12.3	106	2.8
Average		104	14.7	102	15.1	93	9.4

effect. With 1000 units of penicillin, only semen diluted with Ringer's solution at the rate of 1:2.5 showed a slightly positive response.

The addition of penicillin to semen held at room temperature for three hours did not show any beneficial effect on its fertility except for semen diluted 1:2.5 with skimmilk plus 500 units of penicillin.

The better results obtained from the addition of 500 units of penicillin might be due to the inhibition of bacterial activity in semen samples during storage. However, the addition of 1000 units of penicillin may have been an overdose that was toxic to the bacterial flora as well as to the sperm cells.

In general, dilution at the rate of 1:2.5 gave better results than diiution at 1:5.0 under room temperature or 10°C. storage in the presence or absence of penicillin.

Effect of various factors on sperm motility. Storage temperature had the greatest adverse effect on sperm motility, followed by addition of penicillin, diluents used, storage time, and dilution rates (table 5).

In general, normal motility ratings were maintained after storage at 10°C., but not at room temperature. At 10°C., sperm activity apparently slowed down, whereas at room temperaure it was stimulated. Sperms stored at room temperature tended to be

more exhausted than those stored at 10°C. after the three-hour storage period, thereby showing a lower motility rating.

TABLE 5.—*The effect of various treatments on sperm motility*^a

DILUENT	MOTILITY RATING AFTER STORAGE FOR:			
	1 hour without penicillin	3 hours without penicillin	3 hours with 500 units penicillin per ml.	3 hours with 1000 units penicillin per ml.
<i>1:2.5 Dilution at Room Temperature</i>				
Ringer's	4.25	3.25	3.00	2.33
Locke's	3.83	3.75	5.00	2.33
Skimmilk	4.20	2.25	2.25	2.66
<i>1:2.5 Dilution at 10°C.</i>				
Ringer's	4.86	5.00	5.00	4.66
Locke's	4.73	5.00	5.00	5.00
Skimmilk	4.43	5.00	5.00	5.00
<i>1:5.0 Dilution at Room Temperature</i>				
Ringer's	4.00	3.25	3.00	2.66
Locke's	3.83	3.50	5.00	2.66
Skimmilk	4.00	2.25	2.75	2.66
<i>1:5.0 Dilution at 10°C.</i>				
Ringer's	4.66	5.00	5.00	5.00
Locke's	4.57	5.00	5.00	5.00
Skimmilk	4.40	5.00	5.00	5.00
<i>Undiluted at Room Temperature</i>				
	3.33	4.00	4.00	4.66
<i>Undiluted at 10°C.</i>				
	4.12	5.00	4.75	5.00

^a Average from final motility ratings of several samples, initial motility in all cases are the same (5).

The addition of 1000 units of penicillin per ml. of diluted semen aggravated the adverse effect of room temperature on motility and possibly on viability of sperms. This level of penicillin concentration appeared to have a toxic effect on sperms.

Motility ratings of semen diluted with Locke's solution and stored for three hours at 10°C. and at room temperature were higher than those of semen diluted with either skimmilk or Ringer's solution. In fact, motility of stored semen diluted with Locke's solution plus 500 units per ml. of penicillin was equal to that of fresh semen. This could be due to the presence in Locke's solution of

dextrose, a possible source of energy. In bull semen, Nazareno (1958) reported that the survival time of sperms was improved by the addition of dextrose to the diluents.

After one hour storage at room temperature, undiluted semen gave higher motility rating than diluted semen, possibly because dilution provided a wider field for movement of sperms in the media, thus giving them more opportunity to get exhausted faster. However, after three hours storage at the same temperature, the reverse was observed, showing that room temperature had a more exhausting effect on the sperms in the undiluted than in the diluted media. Another possible explanation is that the greater the density of sperms, the faster is the accumulation of metabolites in the medium. In maintaining semen motility, no difference was observed between dilutions of 1:2.5 and 1:5.0.

Sperm concentration and fertility. The average sperm concentration of ejaculates collected from the cockerels was 4.5×10^9 per ml., with a range of 2.54 to 5.95×10^9 per ml. With an inseminated dose of 0.1 ml. per hen, the average number of sperms inseminated appeared to be about 180 million for the 1:2.5 dilution and 90 million for the 1:5.0 dilution.

Munro (1938) and Polge (1951), in their study of the effect of semen dilution and density on the fertilizing capacity of fowl sperm, concluded that the insemination of 100 million or less sperms decreased the percentage of fertile eggs produced. In similar studies, however, Allen and Skaller (1958) and Wilcox (1958) reported that a high level of fertility was obtained with the insemination of less than 100 million sperms to each hen. The results of this experiment corroborated the earlier findings in that lower fertility was obtained consistently with dilution of 1:5.0 in which the average number of sperms inseminated was less than 100 million.

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